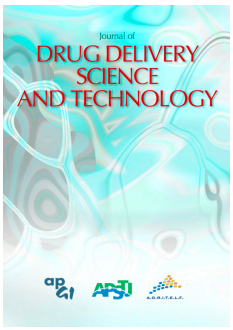
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.



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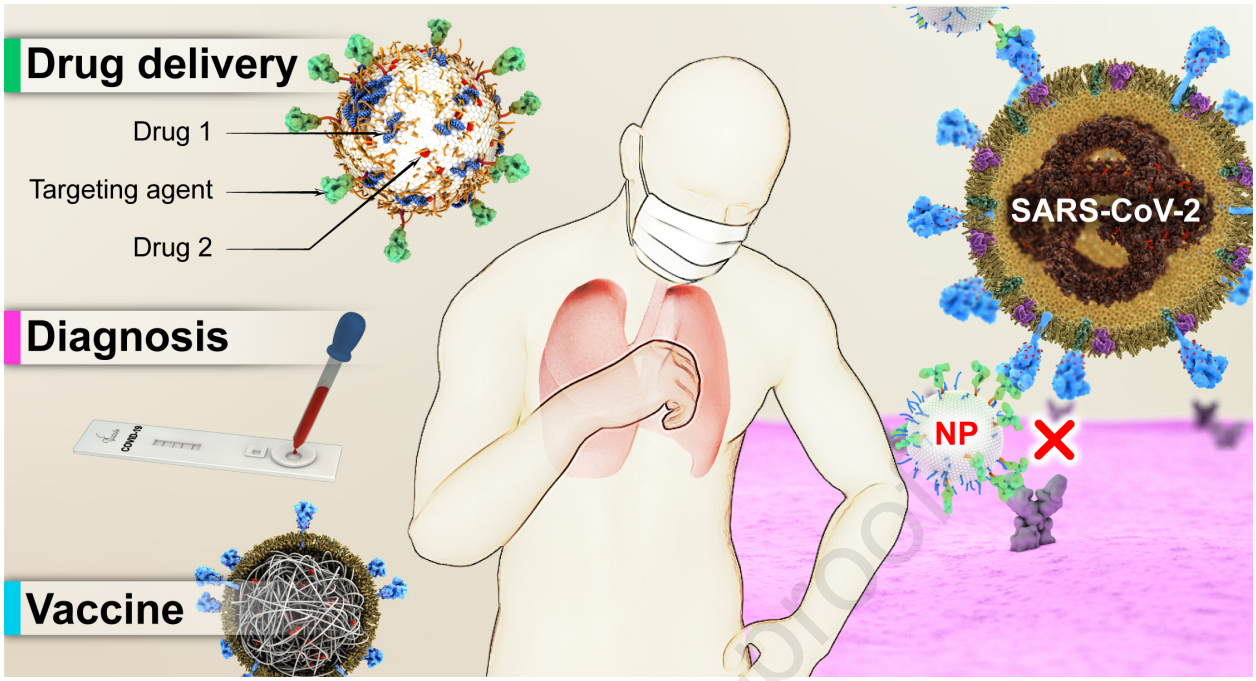

The quest for a better fight: How can nanomaterials address the current therapeutic and diagnostic obstacles in the fight against COVID-19?

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Abstract

The inexorable coronavirus disease 2019 (COVID-19) pandemic with around 226 million people diagnosed and approximately 4.6 million deaths, is still moving toward more frightening statistics, calling for the urgent need to explore solutions for the current challenges in therapeutic and diagnostic approaches. The challenges associated with existing therapeutics in COVID-19 include lack of in vivo stability, efficacy, and safety. Nanoparticles (NPs) can offer a handful of tools to tackle these problems by enabling efficacious and safe delivery of both virus- and host-directed therapeutics. Furthermore, they can enable maximized clinical outcome while eliminating the chance of resistance to therapy by tissue-targeting and concomitant delivery of multiple therapeutics. The promising application of NPs as vaccine platforms is reflected by the major advances in developing novel COVID-19 vaccines. Two of the most critical COVID-19 vaccines are mRNA-based vaccines delivered via NPs, making them the first FDA-approved mRNA vaccines. Besides, NPs have been deployed as simple, rapid, and precise tools for point of care disease diagnosis. Not enough said NPs can also be exploited in novel ways to expedite the drug discovery process. In light of the above, this review discusses how NPs can overcome the hurdles associated with therapeutic and diagnostic approaches against COVID-19.

Keywords: COVID-19; SARS-CoV-2; coronavirus; nanoparticle; nanotechnology; antiviral.

1-Introduction

The 2019 coronavirus disease (COVID-19) has turned into a global threat after its first occurrence in Wuhan, Hubei at the end of 2019. World Health Organization (WHO) declared COVID-19 as a public health emergency of international concern (PHEIC) on 30 January 2020 [1] and with over 226 million cases diagnosed and 4.6 million deaths till now (as of 5:20 pm CEST, 17 September 2021) [2], the disease is still growing unstoppably toward more frightening statistics. The undeniable socio-economic burdens of the disease emphasize the need for more efficacious and safe therapeutics.

Seven types of human coronaviruses (HCoVs) have been identified, which are responsible for respiratory infections [3]. While 4 types of HCoVs cause limiting upper respiratory infections, three of them are responsible for more serious and even lethal diseases such as middle eastern CoV (MERS-CoV), severe acute respiratory syndrome CoV (SARS-CoV), and the new coronavirus. The new coronavirus is officially called SARS-CoV-2 due to the great genome similarity that it shares with the SARS-CoV, responsible for the 2002 SARS outbreak [4]. COVID-19 is associated with manifestations varying from that of flu-like illness such as fever, cough, sore throat, headache, and fatigue to pneumonia. In more severe cases of the disease, it can cause acute respiratory distress syndrome (ARDS), septic shock, and multi-organ failure leading to the death of patients [5].

SARS-CoV-2 is a 50-200 nm enveloped virus and its genome consists of positive-sensed single-stranded RNA (+SSRNA) with ~30 Kilobase pair (Kbp) length, encoding 16 non-structural proteins (NSP) and structural proteins including spike (S), membrane (M), envelope (E) and nucleocapsid (N) [5]. The spike protein has two subunits S1 and S2, where the former is responsible for recognition and binding to host angiotensin-converting enzyme (ACE)2 receptors and the latter facilitates the virus envelope fusion with the host cell membrane [6]. The key roles

of S protein in viral entry to the host cells make it a great therapeutic target for developing antiviral agents against SARS-CoV-2.

Currently, there is a myriad of clinical trials and studies evaluating the medications such as antiviral drugs, immunosuppressive agents, and antibodies [7]. Yet, no medication has been approved for the treatment of COVID-19. The only antiviral drug that has gained Emergency Use Authorization (EUA) from the U.S. Food and Drug Administration (FDA) is the antiviral agent remdesivir [8]. Also, recently, FDA has issued an EUA for the drug Actema (tocilizumab), only for hospitalized adults and more than 2 years old pediatric patients, under certain treatment conditions[9]. On June 15, 2020, FDA rescinded the EUA of Chloroquine (CQ) and hydroxychloroquine (HQC), due to the lack of efficacy in shortening the recovery time of patients or mortality ratio, and also arrhythmia and other adverse effects associated with their administration [10].

There are also significant efforts in developing novel anti-SARS-CoV-2 vaccines [7] and diagnostic tools [11]. Different types of vaccines including subunit vaccines, RNA/DNA-based, attenuated virus vaccines, and virus-like particles (VLPs) are being developed for COVID-19. In the diagnosis field of COVID-19, the reference test is reverse transcription-polymerase chain reaction (RT-PCR). However, these tests are slow, not available for everyone, and not error-free [12]. In fact, they have been associated with some false negative/positive results, particularly in the early stages of the disease. Hence, efforts in designing novel point of care diagnostics, which are available, reliable, precise, inexpensive, simple, and rapid are timely and welcome.

Nanomaterials particularly, Nanoparticles (NPs) are being used in various fields of medicine due to their exceptional characteristics [13]. The unprecedented breakthrough of NP-based therapeutics goes back to 1995, when the first nanomedicine named Doxil was approved by the FDA to treat

ovarian cancer and acquired immunodeficiency syndrome (AIDS)-related Kaposi's sarcoma [14]. Doxil was a liposomal doxorubicin and the key aspects of its success were the high loading efficiency due to the active loading, passive targeting of tumor microenvironment (TME) due to the enhanced permeability and retention (EPR) effect, and the enhanced circulation half-life owing to its PEG coating [15]. In the following year, another liposomal nanomedicine named DaunoXome was FDA approved to treat human immunodeficiency viruses (HIV)-associated Kaposi sarcoma [14]. Since then, nanomedicine has continued to introduce over 50 FDA-approved nanoparticle therapies and diagnostics to the world [16], which highlights the great potential of this category of therapeutics.

NPs are used in therapeutic modalities as drug/gene and protein delivery vehicles [17]. They have also been used as antigen delivery vehicles in cancer immunotherapy and in infectious diseases. NPs size, charge, structure, and surface chemistry can be tuned for specific purposes; from loading to release of the cargo, there are a variety of strategies that can be chosen according to the therapeutic goals [13]. For instance, the drug can be encapsulated in the NP or conjugated on the surface of it, or NPs can have multi compartments or layers to enable co-delivery of hydrophobic and hydrophilic molecules in one platform. NPs can be conjugated with certain moieties that enable the tissue-specific delivery, or their size and charge can be designed so that the maximum lymphatic delivery and cross-presentation occurs in vaccine platforms [18]. NPs can enable control over the drug release profile providing sustained or stimuli-responsive release of the cargo [13] and also the endosomal escape of gene and antigens [17]. Besides, exceptional characteristics of some NPs can be deployed in colorimetric and electrochemical assays used for diagnostic purposes [19].

The mentioned characteristics of NPs have paved the way for developing more efficacious and safer therapeutics and diagnostics for various infectious diseases, particularly viral infections such as influenza, hepatitis, AIDS, and herpes simplex [20]. Also, there have been very recent efforts of deploying NPs in the fight against COVID-19, applications ranging from self-protection tools to vaccines or even diagnostic devices [21]. In the light of the abovementioned facts, this review will discuss how exclusive characteristics of NPs can be exploited as a solution for existing challenges in the therapeutic and diagnostic means in COVID-19.

2- Therapeutic approaches in COVID-19

There are two stages during the COVID-19 disease; the first stage is the "viral response phase", which occurs when the body reacts to the viral infection by subsequent cellular and humoral immune response. This stage occurs early and has mild symptoms that can be managed with antiviral agents or "virus-directed" therapeutics [22]. Although the proportionate immune response is mandatory to fight the virus, the extensive and unstoppable immune response can exacerbate the condition by imposing collateral damage [23]. In fact, the more lethal stage of the disease can be the "exaggerated host response phase" in which inhibition of immune response by "host-directed" therapeutics is essential [24].In the following, the potential role of NP-mediated drug delivery (NPMDD) and its advantages in each therapeutic regimen is discussed. The summary of the potential advantages of NPMDD in COVID-19 can be found in Figure 1.

2.1 Virus-directed therapeutics and the potential role of NPs as delivery vehicles

In this section, the first category of medicines in COVID-19, named "virus-directed" therapeutics are discussed in three subcategories of antiviral agents, small interfering RNAs (siRNAs), and neutralizing antibodies (nAbs). In each category of therapeutics, their shortcomings, and how NPMDD can overcome the associated challenges are discussed.

2.1.1 Antiviral agents

Antiviral therapeutics used against COVID-19, target different stages of the virus life cycle from inhibition of viral binding to ACE2 receptors to virus transport and post-entry events such as replication, transcription, translation, and virus assembly and release. Among the antivirals that target virus RNA replication is remdesivir, an adenosine nucleoside triphosphate analog, which is an RNA dependent RNA polymerase (RdRp) inhibitor [25] . Remdesivir is the only antiviral drug that has gained EUA from the FDA so far. However, the efficacy and safety of antivirals such as Favipiravir, Tenofovir, Lopinavir/Ritonavir, etc., are also under investigation [22]. Furthermore, there have been recent advances in the use of peptides in treating COVID-19 [26]. Some peptides are known to inhibit various phases of the SARS-CoV-2 life cycle. For instance, in a study, it was revealed that short peptides derived from NSP10 exert an inhibitory effect on viral 2'-O-methyltransferase activity, as NSP10 acts as an essential trigger to activate the 2'-O-methyltransferase activity of NSP16 [27]. In another study, the therapeutic potential of a nucleopeptide in treatment of COVID-19 was proposed based on its interactions, investigated in docking studies, with 3CLpro, the papainlike protease, the coronavirus helicase, and the RdRp targets in SARS-CoV-2 [28].

The oral bioavailability of the antiviral molecules and their ability to overcome the biological barriers such as mucus and blood brain barrier (BBB), in case the virus is detected in the central nervous system (CNS), play essential roles in their enhanced efficacy. Many of the mentioned antiviral agents now exploited in COVID-19 have been previously used against a variety of viruses such as the HIV, herpes simplex viruses (HSV), hepatitis B (HBV) and C (HCV) viruses, H1N1 influenza virus, etc. [20]. In such diseases, NPs have been exploited to enable efficient delivery of antivirals via overcoming the biological barriers in the body, thereby enhancing the therapeutic

outcome of these molecules. For instance, the oral bioavailability of efavirenz, a poorly soluble drug, in rabbits has increased by more than 2 folds via nanosuspension based formulation, which was able to enhance solubility, dissolution rate, and permeability of the drug molecules [29]. Moreover, NPs can improve the physicochemical characteristics of antiviral agents while decreasing their needed doses. In this vein, they can subsequently reduce the risk of adverse effects and resistance of antiviral agents. For instance, lamivudine loaded Eudragit E100 laden buccal films used for HIV therapy in pediatrics showed dose sparing effect, and thus reduced toxicity in pediatrics. Besides, the formulation enhanced patient compliance due to the convenient dosage form, which enabled the sustained release of drug for 2 days [30]. NPs role in respiratory viral infections such as H1N1 has also been evaluated; novel anti-H1N1 therapies were designed to defeat multidrug-resistant disease types by deploying decorated Se NPs with amantadine [31] and also oseltamivir [32], which showed promising results in vitro. Also, diphyllin and bafilomycin loaded Polyethylene glycol (PEG)-PLGA diblock polymers were used against influenza [33]. These formulations enhanced the therapeutic index of these agents by 3 and 5 fold, respectively, and also enabled the sustained release of them for more than three days. Low blood brain barrier (BBB) permeability of drugs had also been successfully addressed via different NPs including solid lipid NPs (SLNs), LNPs, copolymers, NP conjugates, etc. [20]. Some NP-based platforms are also approved by FDA, or other organizations for administration in viral infections including PegInteron (in HCV), Pegasys (in HBV, HCV), and VivaGel (in HIV, HSV) [20].

In the light of the aforementioned knowledge gained by previous viral infections by utilizing NPs, there has been recent advances in tackling the challenges associated with antiviral therapeutics against COVID-19.

In a study, inhalable liposomes containing HQC were used to enhance the PK profile of the HQC. This novel formulation can increase HQC efficacy and safety by enhancing its lung exposure and half-life in lungs ,while reducing its systemic exposure [34]. Another study, also evaluated the potential of magnetic delivery of HQC, by a Fe_3O_4-based biocompatible nanocarrier [35].

Also, there are different hypotheses exploring the potential of nanoparticulate drug delivery systems in COVID-19. For instance, nanostructured lipid carriers (NLCs) are proposed to be good intra-pulmonary delivery vehicles for salinomycin, as a potent antiviral agent against SARS-CoV-2. NLCs advantages are regarded as noninvasive means of administration, direct pulmonary delivery, more efficacy by avoiding first pass metabolism and rapid drug absorption and also reducing the side effects [36]. Also, liposomal nanocarriers are proposed to be effective in enhancing the solubility of ivermectin and also reducing its toxicity as a therapeutic candidate against SARS-CoV-2 [37]. Favipiravir is another antiviral agent that has gained attention to treat mild or moderate COVID-19 patients [38]. There are some hypotheses, which explore the potential of NPs as vehicles for favipiravir. A study proposed the dual respiratory delivery of favipiravir and Tocilizumab via protein-lipidic nanovesicles [39]. Based on their intermolecular interactions another study proposed the BC_{23}, $B_{12}N_{12}$, and $CB_{11}N_{12}$ nanocages as promising carriers for favipiravir [40]. The inhaled NO therapy also showed enhanced antiviral activity and also ventilation perfusion in the lungs of COVID-19 patients. NO antiviral activity is attributed to its role in inhibition of S protein fusion and viral replication of SARS-CoV-2. The therapeutic efficacy of NO therapy can be augmented by use of NO donors with nanomaterials, which can increase the antiviral activity of NO and also enable sustained and localized release of it. For this purpose, chitosan NPs can be promising candidates due to their mucoadhesiveness, biocompatibility and their former application in pulmonary drug delivery [41].

2.1.2 Small interfering RNA (siRNA) therapeutics

During the outbreak of COVID-19 also the idea of targeting the SARS-CoV-2 viral RNA genome via RNA interference (RNAi) technology such as small interfering RNA (siRNA), RNA aptamers, and antisense oligonucleotides got widespread attention [42]. For siRNA therapy, different regions of the virus genome have been used as the target such as SARS-CoV-2 M, N, and E genes as well as RNA polymerase and replicase regions of the genome [7]. Despite the great potential of siRNA therapeutics, some hurdles regarding their systemic delivery exits. Previous studies on the effect of NP-based siRNA delivery systems in other diseases such as cancer, viral infections, and ocular diseases highlight the role of NPs in addressing the challenges for more effective siRNA therapy [43]. One of the most important challenges are in vivo stability and specificity of siRNAs to avoid their rapid degradation and loss of function as well as preventing their off-target effects. In this sense, well-engineered and tissue-targeted NPs can overcome these obstacles by protecting the encapsulated siRNA from enzymatic degradations as well as delivery to the target tissue. Besides, NP-based siRNA delivery systems not only can reduce the clearance of siRNAs by the reticuloendothelial system (RES), but also can reduce the immunotoxicity of these therapeutics [43]. Furthermore, NPs can enable the endosomal escape of siRNAs, which is an essential step to prevent the degradation of siRNAs in endosomes [17].The most successful RNAi delivery systems are LNPs with Patisiran/ONPATTRO, the first siRNA therapeutic with FDA approval, and several other candidates in clinical trials [44]. In addition to LNPs, different other types of NPs, including silicon and carbon-based NPs, polymers, dendrimers, metal and metal oxides, and nanocrystals have also been used for this purpose [43]. Inhalable NPs and polymer-based platforms, such as PEG- polyethyleneimine (PEI) NPs have been previously deployed in targeted siRNA delivery to lungs and seem to be promising candidates in COVID-19 [45]. It is noteworthy to mention the

major challenge for LNP-based RNAi therapeutics that is their accumulation in the liver, which hinders their application in COVID-19 [46]. To address this challenge, previous reports showed that the increased concentrations of 1,2-dioleoyl-3-trimethylammonium-Propane (DOTAP) and DLin-MC3-DMA (MC3) in the LNPs are positively associated with their lung-targeting ability, and by adjusting their ratio, one can achieve good lung targeting with LNPs [46]. However, since high concentrations of DOTAP are associated with systemic toxicity, a recent study, designed a LNP platform with lower concentrations of DOTAP for SARS-CoV-2 targeted siRNA-nanoparticle therapy for COVID-19 [47]. The results of this study showed that this platform could target lung efficiently and successfully suppress SARS-CoV-2 in vivo. In light of these, NP-based siRNA delivery systems can be a promising approach to silence the SARS-CoV-2 genome targets to halt the infection.

2.1.3 Neutralizing antibodies (nAbs)

In addition to what has been mentioned, the other important target for developing antivirals against SARS-CoV-2 is S protein due to its essential role in virus attachment to host ACE2 receptors. Neutralizing antibodies (nAbs) against S protein or its receptor-binding domain (RBD) are being designed and evaluated [7]; also, recently, the first Ab against S protein entered phase I clinical trial [48]. Besides, peptide inhibitors against SARS-CoV-2 are being designed by the aid of molecular dynamics (MD) simulations [49]. These molecules inhibit the priming of S protein by transmembrane protease serine 2 (TMPRSS2), an essential step for the fusion of viral and host cell membranes. In this context, nanomaterials can provide a unique characteristic of multivalent binding to the virus receptors. These nAbs or peptide inhibitors can be conjugated on the surface of NPs not only to be protected, but also to increase the chance of interaction with virus receptors to further inhibit the viral attachment and fusion to host cells (Figure 2). In an interesting study, a

nAb-conjugated photothermal NP was designed to capture and inactivate SARS-CoV-2 [50]. This NP takes advantage of surface nAbs, which enable targeting, capturing, and inhibition of SARS-CoV-2 entry to ACE2- expressing host cells and a semiconductive polymer, which shows excellent photothermal effects upon light emitting diode (LED) light excitation. This study highlights the multi-functional approach of utilization of NPs for complete inactivation of SARS-CoV-2.

2.2 Host-directed therapeutics and the potential role of NPs as delivery vehicles

The second mode of therapeutics is host-directed drugs. These therapeutics are used to dampen the harmful exaggerated host response, which can lead to ARDS. In this phase infiltration of immune cells and the cytokine release syndrome (CRS) lead to dysfunctional immune response and production of non-neutralizing Abs [51]. Host-directed therapeutics are generally immunosuppressive agents such as glucocorticoids (GCs), monoclonal antibodies (mAbs) against interleukin (IL)-6 (tocilizumab), IL-1 receptor blocker (anakinra), tumor necrosis factor (TNF)-α inhibitor (etanercept), and JAK inhibitors (baricitinib) [52, 53]. Since the heightened inflammation in the lung is one of the major causes of ARDS, the delivery of the immunosuppressive drugs to the inflammatory lung can be one of the most effective strategies. A large body of evidence shows that inflammatory tissues release the mediators that induce the EPR effect [54]. It is widely known that NPs can passively accumulate in the EPR-enhanced target sites as they do in the TME. However, the inflammatory tissue differs with TME in the presence of a functional lymphatic drainage system, in this case, macrophage uptake can enable the retention of NPs in inflammatory tissues [54]. Many of the host-directed therapeutics are similar to those used against rheumatoid arthritis (RA). Since NPMDD had shown promising results in RA [55, 56], exploiting NPs for this purpose can also provide benefits in the COVID-19 emergency. For instance, long term delivery of etanercept in RA was achieved via temperature modulated electrostatic interaction between the

positively charged etanercept and the negatively charged amphiphilic co-polymer, succinylated pullulan-g-oligo(L-lactide) (SPL) [57]. The nanocomplex showed the increased stability of etanercept as well as enhanced pharmacokinetic profile. Also, the successful delivery of Tocilizumab was achieved by the Hyaluronate-Au NP/tocilizumab complex [58]. Since mAbs and proteins need protection from degradation in the systemic circulation, mAb-conjugated NPs or protein/mAb- loaded NPs can be excellent candidates for safe delivery of these therapeutics. Furthermore, the safety issues regarding the immunogenicity or toxicity of these agents can be addressed via NP-based approaches.

Another group of medications in this category is Janus Kinase (JAK) inhibitors. The intracellular delivery of JAK inhibitors, and enhancing their bioavailability can be achieved via NPMDD. For instance, co-polymer-based NPs were used for the simultaneous delivery of two JAK inhibitors in non-small cell lung cancer (NSCLC) to overcome the resistance to therapy [59]. And also other co-polymer based NPs were used to improve the bioavailability of baricitinib [60].

NPs also have been used for the delivery of corticosteroids in different diseases such as cancer, RA, and neuroinflammatory diseases [61]. Different NPs have been employed for such purposes including PEGylated liposomes, polymers (micelles and drug conjugates), inorganic scaffolds, and hybrid NPs. NPs passive accumulation in the inflammatory tissues also enables the significant reduction in the needed- dose of GCs, leading to improved efficacy, specificity, and tolerability of these therapeutics. Besides, NPs can enhance the physicochemical characteristics and also the half-life of GCs [61]. Furthermore, they can also provide a depot of GCs in the body by controlled delivery platforms.

Mesenchymal stem cells (MSCs) therapy also was proved to be efficacious treatment modality in COVID-19 pneumonia patients [62]. This effect is attributed to the immunomodulatory roles

associated with MSCs, which attenuate the ARDS. However, lack of in vivo stability of MSCs and their tendency to aggregate hinders their application. On the other hand, it has been reported that MSC-derived exosomes as cell-free therapeutics can provide the therapeutic efficacy of MSCs without having the mentioned problem. The biocompatibility and cell targeting ability of these exosomes also makes them ideal candidates for drug delivery in COVID-19 [63].

2.3 Concomitant delivery of multiple therapeutics in combinational regimens

In the past, monotherapy with antiviral agents like ribavirin against SARS-CoV and MERS-CoV showed limited efficacy in patients and also provoked safety concerns regarding hemolysis collateral effects [64]. In this sense, combinational therapies based on the administration of ribavirin with ritonavir and lopinavir were evaluated and showed promise in SARS patients [65]. Furthermore, different combinations of interferons (IFNs) with ribavirin or lopinavir/ritonavir were evaluated in SARS and MERS patients [66-68]. Besides, combinations of virus-directed therapies with host-directed therapies have also been investigated including combinations of IFNs with immunosuppressive agents such as corticosteroids and mycophenolate mofetil in MERS patients [69, 70].

Different combinations of therapeutics were also evaluated in COVID-19 patients, which yielded promising results. For instance, a triple combination of IFN beta-1b, lopinavir/ritonavir, and ribavirin was compared with lopinavir/ritonavir therapy in COVID-19 patients with mild to moderate symptoms [71]. The result revealed that the combination therapy was more effective in alleviating the symptoms and shortening the hospitalization duration. Other combinations such as darunavir/cobicistat [72] and emtricitabine/tenofovir (NCT04334928) were also investigated in COVID-19. In this context, NPs can offer an exclusive advantage of concomitant delivery of drugs. Concomitant delivery of therapeutics has several benefits such as reducing the drug administration

frequencies, which helps to improve the compliance of patients and lowers the risk of resistance to treatment regimen while increases the efficacy and safety of the therapy.

Nanomaterials are excellent candidates for such purpose and there are plenty of strategies that enable simultaneous delivery of drugs by a single nanocarrier [73]. Different therapeutics can also be loaded in different compartments of NPs so that even non-compatible molecules can be co loaded in an NP and also by tailoring the physicochemical characteristics of each compartment or layer, the release profile of drugs can be controlled so that the therapeutic concentration of each medicine remains adequate during a long time. Best examples of concomitant delivery of antivirals via NPs can be found in attempts being made in combinational therapies against HIV. For instance, PLGA NPs were used for delivery of efavirenz, lopinavir, and ritonavir, with entrapment efficiency of around 80% for each drug [74]. Besides, lactoferrin NPs were used for the delivery of zidovudine, efavirenz, and lamivudine. Another study in primates revealed that LNPs simultaneously carrying lopinavir, ritonavir, and tenofovir significantly enhanced intracellular concentrations in lymph nodes and blood and were able to sustain the drug concentrations in plasma for up to7 days [75]. These studies emphasize the great potential of NPS to be deployed in combinational regimens in COVID-19.

2.4 Vulnerable organs to SARS-CoV-2 and the potential role of targeted drug delivery by NPs

The major and primary target of SARS-CoV-2 is the respiratory system, where there is a high expression of ACE2 receptors [76]. The abundant ACE2 receptors existing in lung and bronchial branches cells provide the binding site for SARS-CoV-2, which can lead to the clinical manifestations of the disease such as pneumonia and in more advanced cases, ARDS. By considering this, pulmonary delivery of therapeutic agents against SARS-CoV-2 can be a great

option for a successful treatment. In this sense, NPs can be exploited as promising tools for such a purpose. Pulmonary delivery of drugs by NPs can increase the concentration of drugs in the lung and reduce their side effects. Furthermore, the sustained release of drugs from NPs in the lungs can provide a depot of therapeutics, which results in enhanced patient compliance by reducing the dose frequency. For instance, administration of amikacin-loaded SLNs by micro sprayer in rats increased the drug concentration in the lungs, highlighting the potential of pulmonary delivery of amikacin loaded SLNs in cystic fibrosis patients [77]. Also, the amikacin liposome inhalation suspension Arikayce (®) (Insmed, NJ, USA) got FDA approval in 2018 for the treatment of mycobacterium avium complex (MAC) lung disease [78]. Many other antibiotics including itraconazole, voriconazole, ciprofloxacin, anti-tuberculosis drugs, etc., have been formulated for pulmonary delivery by different NPs [79].

Recently, an inhalable liposomal drug delivery system was utilized to enhance the efficacy and safety of HQC in COVID-19 in a pre-clinical study [34]. Another study, evaluated the intra-pulmonary delivery of remdesivir by nanostructured aggregates using thin film freezing[80]. Also, there are hypotheses about intra-pulmonary delivery of salinomycin and NO-releasing nanomaterials in COVID-19 [36] [41].

Although the respiratory system is the primary target of SARS-CoV-2, the virus can spread to other organs with a high density of ACE2 receptors in severe cases. For instance, the cardiovascular system including heart and endothelial cells also express high amounts of ACE2 receptor and can be potential targets for the invasion of SARS-CoV-2 [81]. Besides, ACE2 and TMPRSS2 are also found abundantly in the gastrointestinal tract (GIT) including the duodenum, small intestine, pancreas, and liver, which makes these organs susceptible to SARS-CoV-2 [76]. Furthermore, the central nervous system (CNS) is also not immune to the attack of SARS-CoV-2

[82]. The blood brain barrier (BBB), which protects CNS from the invasion of pathogens, can get harmed as a result of the CRS triggered in response to SARS-CoV-2, leading to its increased permeability to the virus. Furthermore, sensory or motor nerve endings can provide a pathway for the migration of the virus to CNS. In particular, olfactory nerves can make a direct passage from the nasal cavity to CNS, enabling the direct invasion of SARS-CoV-2 to CNS [82]. In each of the mentioned cases, NPMDD approaches can also be helpful. Since NPs have been successfully used for targeting the cardiovascular system [83], liver [84], GIT [85], and CNS [86].

The reproductive system also expresses high amounts of ACE2 receptors particularly in the placenta, uterus, and fetal interface of pregnant women [87]. There is evidence regarding the increased susceptibility of pregnant women to SARS-CoV-2 as a result of the changes in the anatomical structure of the respiratory system, hormones, immune system, and also upregulation in ACE2 receptors during pregnancy [88]. In this condition, adverse effects associated with anti-COVID-19 therapies on fetus becomes of great concern. NPs can be implemented to improve the efficacy and safety of drugs in pregnant women. In addition to the general benefits that NPs can provide by the delivery of antivirals, NPs can have additional benefits as fetus-safe delivery systems. NPs characteristics including size and charge can be tailored so that their affinity to maternal target organs be increased while the possibility of their interaction with the placenta is minimized [88].

3- Vaccine development in COVID-19 and role of NPs as vaccine platforms

There has been great endeavor in designing prophylactic and therapeutic vaccines for COVID-19. According to WHO COVID-19- Landscape of novel coronavirus candidate vaccine development worldwide, there are currently 105 candidate vaccines in clinical evaluation against SARS-CoV-2 and 184 vaccines in the preclinical stage. 84 percent of current COVID-19 vaccines in clinical

phase are administered via injection (84%), mostly intra muscular, and 65% of them require 2 doses to obtain the desirable immunity, while 14% require one dose and only 1% require 3 doses.

One of the most important areas of implementing NPs for prophylaxis of COVID-19 is developing mRNA-loaded LNPs since the two FDA-approved vaccines used for mass vaccination belong to this category, which will be discussed in section 3.3. Among other vaccines, there is a non-replicating viral vector named Ad26.COV2.S with EUA, developed by the Janssen Pharmaceutical Companies (Johnson & Johnson group), that has shown good safety, immunogenicity, and efficacy after a single dose [89].There are two non-replicating viral vectors being used for mass vaccination in countries other than the U.S. The Vaxzeveria is developed by University of Oxford/AstraZeneca and Gam-COVID-Vac/Sputnik V vaccine, is produced in Russia by the Gamaleya Research Institute [90]. Vaxzeveria, is an adenovirus-based non-replicating vector, which showed an efficacy of 62-90% in phase 3 clinical trials [91], and Gam-COVID-Vac/Sputnik V vaccine is based on the combination of the two replication-deficient adenoviruses type 26 (rAd26) and rAd5, with genetic information for expression of the full-length glycoprotein S of SARS-CoV-2 [92]. There are also several other vaccines in different stages of clinical trials that have been comprehensively reviewed elsewhere [90].

In the following, the beneficial role of NPs in developing each kind of vaccine against SARS-CoV-2, which fall into different categories as inactive or live-attenuated viruses, virus-like particles (VLPs), RNA and DNA-based vaccines, and protein subunit vaccines, is discussed.

3.1 Subunit vaccines

Subunit vaccines consist of protein or glycoprotein molecules of a pathogen, that can induce a protective immune response, and account for the 31% of current COVID-19 candidate vaccines in

clinical phase. The S, E, M, and N proteins of SARS-CoV-2 are potential antigens that are being used in subunit vaccines of COVID-19 [7]. However, these antigens are prone to degradation and loss of activity in the biological environment. Furthermore, without an appropriate delivery system, these antigens are not likely to accumulate in their site of action, which is lymph nodes. Moreover, the chance of the uptake of particulate antigens by antigen-presenting cells (APCs) is much higher than that of soluble antigens [93]. In this line of thought, NPs are the best candidates for the delivery of subunit vaccines due to their exclusive characteristics that can guaranty successful antigen delivery. NPs have been widely used for this purpose in various infectious diseases and cancers [18, 94]. NPs are excellent vehicles for the delivery of antigens since not only they can enhance the in vivo stability of antigens, but also can deliver antigens effectively to lymph nodes, where their particulate structure will further facilitate the internalization of antigens by APCs. Followed by the uptake of antigen laden NPs, APCs will mature and cross-present the antigen via MHC1 complex on their surface, a signal which can activate $CD8^+$ cells to activate Ag-specific cytotoxic T cell-mediated immunity [18]. In addition to the role of NPs in carrying the viral antigen, the antigen itself can be assembled to a nanoparticulate structure. For instance, a self-assembling polypeptide NP vaccine based on the repetition of the heptad repeat region (HRC) of SARS-CoV S protein was designed. The self-assembly vaccine could successfully trigger anti-SARS antibodies even in the absence of adjuvants. The potent immunogenic effect of the system was due to the repetitive display of the epitope and size of the particle, which ensured its successful uptake and presentation by APCs [95]. Another study evaluated the role of RNA as a molecular chaperon to enable the folding of monomeric antigens of MERS-CoV RBD to high order and more immunologically relevant conformations to form a potent self-assembly NP vaccine [96]. Even an NP-base vaccine without any viral antigens was found to exert an immuno-prophylactic effect on

mouse-adapted SARS-CoV via the prior development of inducible bronchus-associated lymphoid tissue (iBALT) in the lung. The reported NP was protein cage NP (PCN) derived from the small heat-shock protein (sHsp 16.5) of the hyper-thermophilic archaeon *Methanococcus jannaschii* that effectively cleared respiratory viruses and also succeeded to avoid the host exaggerated immune response and further lung damage [97].

Another important moiety in vaccines is adjuvant, which can trigger a more potent immune response and lower the needed antigen dose [98]. Some NPs intrinsically can act as adjuvants via triggering the immune response, however, the efficacy of NPs as adjuvants should be meticulously evaluated. For instance, in a recent study [99], a candidate vaccine based on the recombinant S protein of SARS-CoV was designed. The subunit vaccine was used in combination with two adjuvants separately since the adjuvant-free formulations could not induce a potent immune response and caused lung eosinophilic immunopathology (LEI). Accordingly, the first system was Au NPs conjugated with the S protein in which Au NPs were used because of their intrinsic immuno-stimulatory properties, and the other adjuvant was a Toll-like receptor (TLR) agonist. Results showed that although Au NP based vaccine was able to induce a strong Ab response, it failed to prevent the LEI. On the other hand, the TLR agonist-adjuvanted vaccine could both induce a strong immune response and successfully abate the LEI. This study reveals that relying on the intrinsic immuno-stimulatory role of NPs is not always promising. Yet, another major benefit of NP-based antigen delivery is the possibility of concomitant delivery of adjuvants along with antigens, which has proved to be a promising strategy in previous attempts in other infections. Contrary to what was mentioned regarding the simultaneous delivery of antigen and adjuvant, some studies shed light on the fact that co-delivery of antigen and adjuvant in the one-and-the-same NP or via physical linking are not the only ways to obtain promising results. This notion

enabled the development of Matrix formulations that are saponin-based homogenous and stable NPs that act as adjuvants and can be used in conjunction with antigen-loaded NPs. Matrix M adjuvant is the Novavax patent, which is more tolerable than the primary formulation and can recruit APCs to the injection site and facilitate antigen presentation in local lymph nodes. In 2017, Novavax reported a MERS-CoV S NP vaccine, which was used with Matrix M1 adjuvant in mice [100]. The combination was able to produce an effective immune response by producing anti-S protein neutralizing Abs and by blocking the infection in the mice. Also, on February 26, Novavax announced the development of a novel COVID-19 vaccine based on coronavirus S protein NPs along with the Matrix-M adjuvant, which is in the Phase 3 clinical phase. In this vaccine, the full length (FL) spikes expressed and purified from insect cells, were formulated in 0.01% (v/v) polysorbate 80 (PS 80) detergent [101]. PS 80 self-assembles into nanoscale micelles and encloses as many as 14 trimers of FL spikes in its micellar cores. Novavax's proprietary Matrix-M™ adjuvant contains two 40-nm-sized particles with individual saponin fractions purified from the tree *Quillaja saponaria* Molina, combined with cholesterol and phospholipid [102]. The Matrix-A particles consist of Fraction-A, which is the weaker and well-tolerated saponin. Matrix-C particles are made of Fraction-C, the highly active saponin. This mixture of two individual nanoparticles significantly improves both adjuvant activity and safety profile.

A list of NP-based subunit vaccines that are under clinical and pre-clinical investigation against SARS-CoV-2 can be found in Table 1.

3.2 Synthetic virus-like particles (sVLPs)

Virus-like particles (sVLPs) account for the 5% of the current COVID-19 candidate vaccines in clinical phase. NP-based sVLPS are generally like antigen-laden NPs and the difference is in the antigen position in NP. In antigen-based NPs, the virus antigen is loaded into the NPs, while in

sVLPs antigen is placed on the surface of NPs. Incubation of NPs with specific viral proteins can maintain sVLPs, which resemble the natural viral particles. One of the most important features of NPs is that their size can be tailored to resemble the virus while they have viral antigens on their surface. These sVLPs will be able to stimulate the immune response in the body, while they are generally harmless because they lack the virus replication system. Coronavirus structural proteins, especially the spike protein can be a potential model antigen for designing vaccines. For instance, Au NPs (100 nm) with a protein corona of avian coronavirus spike protein was used for vaccination in an avian model of coronavirus infection [103]. These sVLPs showed superiority over free proteins in antigen delivery and subsequent immune response of antibody secretion and T-cell response leading to the overall better therapeutic outcome in vivo. The VLPs even outperformed the inactivated virus in antiviral protection.

Alike antigen-based vaccines, sVLPs can also co-deliver adjuvants to impose a more potent immunity (Figure 3). Based on this approach, Lin et al.,2019, designed a viromimetic vaccine against MERS-CoV; in their platform, stimulator of interferon genes (STING) agonist as adjuvants was loaded in hollow polymeric NPs whose surface was coated with the MERS-CoV RBD antigens [104]. Consequently, the morphology of RBD-coated STING agonist loaded polymeric hollow NPs resembled the original virus. Altogether, the NPs could deliver antigens alongside adjuvants to lymph nodes where they managed to release the latter in a pH-dependent manner. Thus, they enabled significant local immune activation and lowered systemic reactogenicity. This safe and efficient vaccine was able to trigger potent humoral and cellular immune response without the occurrence of LEI.

3.3 RNA or DNA based vaccines

mRNA based vaccines have been widely used in a variety of diseases such as cancer and infectious diseases. These vaccines also encompass a considerable portion (17 %) of developing vaccines, which are under clinical trials for COVID-19. The mRNAs developed against MERS-CoV and SARS-CoV mostly encode the full-length S, S1, S2 protein, or RBD. Other mRNAs also encode other structural proteins of the virus such as E, M, and N [7]. These mRNAs followed by cellular uptake and triggering the cellular machinery, are translated to the functional viral proteins that can trigger immunity. To protect these highly sensitive molecules from degradation by extracellular RNases and also to enable their effective intracellular delivery, NPs can be used as appropriate tools (Figure 4). Also, to stimulate a more potent response, the idea of combining multiple mRNAs into a single vaccine exists. In this case, concomitant delivery of mRNAs via a single carrier can be promising. Different NPs have been used for the delivery of mRNAs such as dendrimers, cationic polymers including PEI and chitosan, nano-emulsions, liposomes, and lipid NPs. lipid nanoparticles (LNPs) are the most used NPs as mRNA delivery vehicles that consist of ionizable lipid, phospholipid, PEG-lipid as the half-life increasing moiety, and cholesterol as the stability-enhancing component [105]. The structure of LNPs including their multilamerallity, which can be tailored by using cholesterol derivatives, plays a pivotal role in enhanced gene delivery of these NPs [106].

Two important vaccines of this category that are currently in the Phase 4 clinical trial are the Moderna/ the National Institute of Allergy and Infectious Diseases (NIAID) and BioNTech/Fosun Pharma/Pfizer vaccines. These vaccines are the first mRNA vaccines that have gained FDA approval so far and underscore the significant role of using nanomaterials to enhance vaccines' safety and efficacy. Moderna/NIAID have developed the mRNA-1273 vaccine against SARS-CoV-2, which composes of cationic LNPs as carriers of mRNA and shows an efficacy of around

94% among 30k participants [107]. Moderna/NIAID vaccine has been approved for adults ages 18 or older in the U.S. The BNT162b2, developed by the BioNTech/Pfizer/ Fosun Pharma group, is also an mRNA-based LNP vaccine, which showed an efficacy of 95% among more than 43k participants enrolled in its phase 3 clinical trial [108]. Pfizer vaccine is approved for adults ages 16 and older in the U.S. with EUA for ages 12-15.

Also, a thermostable LNP-encapsulated mRNA vaccine named ARCoV that encodes RBD of SARS-CoV-2 is being evaluated in phase 3 clinical trial. Intramuscular administration of this vaccine triggered the production of neutralizing Abs against the virus and promoted a T helper 1-biased cellular response in mice and non-human primates (Figure 4) [109]. Furthermore, immunization by this vaccine provided successful protection against the challenge of SARS-CoV-2 in the mouse model. This liquid vaccine can be kept at room temperature for a minimum of 1 week. A list of LNP-mRNA COVID-19 vaccines in pre-clinical and clinical phases can be found in table 1.

DNA vaccines are also being designed and tested in COVID-19. These vaccines also face some of the challenges of mRNA-based vaccines such as targeted delivery to desired cells (such as APCS) and the endosomal escape, required to release the mRNA or DNA to the cytoplasm [110]. Although plasmid DNA is a more stable molecule than mRNA, DNA-based vaccines face the vital challenge of entering the cell's nucleus, which is absent in mRNA-based vaccines [110]. There are different strategies for effective delivery of DNA vaccines such as electroporation, sonoporation, and nanomaterial-based non-viral gene delivery vehicles [111]. Despite the significant success of LNP-based mRNA vaccines in COVID-19, there are no NP-based DNA vaccines, the reason of which can be attributed to their challenge of nuclear localization.

Table 1. NP-based vaccine candidates in clinical and preclinical evaluations mentioned in the WHO COVID-19- Landscape of novel coronavirus candidate vaccine development worldwide (as of June 29, 2021)

Platform	Type of candidate vaccine	Developer	Status
RNA	LNP encapsulated mRNA "mRNA-1273.351. A lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine that encodes for a full-length, prefusion stabilized S protein of the SARS-CoV-2 B.1.351 variant."	Moderna/NIAID	Phase 4
RNA	BNT162b2 (3 LNP-mRNAs), also known as "Comirnaty"	BioNTech/Fosun Pharma/Pfizer	Phase 4
RNA	SARS-CoV-2 mRNA vaccine (ARCoV)	Academy of Military Science (AMS), Walvax Biotechnology and Suzhou Abogen Biosciences	Phase 3
RNA	LNP-nCoVsaRNA	Imperial College London	Phase 1
RNA	CoV2 SAM (LNP) vaccine. A self-amplifying mRNA (SAM) lipid nanoparticle (LNP) platform + Spike antigen	GlaxoSmithKline	Phase 1
RNA	HDT-301: Self-replicating mRNA vaccine formulated as a lipid nanoparticle	SENAI CIMATEC	Phase 1
RNA	LNP-nCOV saRNA-02 vaccine; Self-amplifying RNA (saRNA) encapsulated in lipid nanoparticles (LNP)	MRC/UVRI and LSHTM Uganda Research Unit	Phase 1

RNA	**LNP-mRNA**	**Translate Bio/Sanofi Pasteur**	**Pre-clinical**
RNA	**LNP-mRNA**	**Max-Planck-Institute of Colloids and Interfaces**	**Pre-clinical**
RNA	**LNP-mRNA**	**CanSino Biologics/Precision NanoSystems**	**Pre-clinical**
RNA	**LNP-encapsulated mRNA**	**University of Tokyo/ Daiichi-Sankyo**	**Pre-clinical**
RNA	**LNP-encapsulated mRNA cocktail encoding VLP**	**Fudan University/ Shanghai JiaoTong University/RNACure Biopharma**	**Pre-clinical**
RNA	**LNP-encapsulated mRNA encoding RBD**	**Fudan University/ Shanghai JiaoTong University/RNACure Biopharma**	**Pre-clinical**
Protein Subunit	**Full length recombinant SARS CoV-2 glycoprotein NP vaccine adjuvanted with Matrix M**	**Novavax**	**Phase 3**
Protein Subunit	**SpFN (spike ferritin nanoparticle) uses spike proteins with a liposomal formulation QS21 (ALFQ) adjuvant.**	**Walter Reed Army Institute of Research (WRAIR)**	**Phase 1**
Protein Subunit	**Peptide antigens formulated in LNP**	**IMV Inc**	**Pre-clinical**
Protein Subunit	**S subunit intranasal liposomal formulation with GLA/3M052 adjs**	**University of Virginia**	**Pre-clinical**
Protein Subunit	**Recombinant protein, NPs (based on S-protein and other epitopes)**	**Saint-Petersburg scientific research institute of vaccines and serums**	**Pre-clinical**
Protein Subunit	**NP vaccine**	**LakePharma, Inc**	**Pre-clinical**
Protein Subunit	**RBD protein delivered in mannose-conjugated chitosan nanoparticle**	**Ohio State University / Kazakh National Agrarian University**	**Pre-clinical**

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4- Diagnostic approaches in COVID-19 and potential roles of NPs

In addition to the great need for developing therapeutic approaches against the ongoing COVID-19, developing reliable laboratory diagnostic tools are also among the primary concerns. Different laboratory approaches have been developed for the diagnosis of COVID-19 the most routine of which is RT-PCR. This approach, however, has its hurdles; for instance, it is only done by professional people in laboratories and is also very time-consuming. Furthermore, despite the reliability of this approach, it is not error-free since there has been a report of some false-positive/negative cases, particularly in the early stages of the disease [12]. In this sense, developing novel approaches, which can diagnose the COVID-19 rapidly, easily, and accurately remains a priority. A great effort is being invested in designing NP-based approaches that can help in the diagnosis of the virus via colorimetric, electrochemical, and lateral flow immunoassays. Gold nanoparticles are especially appropriate in this sense due to their exclusive characteristics such as localized surface plasmon resonance (LSPR) shift and thermoplasmonic heat generation ability. In the following the NP-based diagnostic tools used for COVID-19 and other closely-related diseases such as MERS and SARS, are discussed.

4.1 Virus detection

One of the important diagnostic approaches is the detection of a specific target of viral antigens, proteins, or genome, and different studies are reporting the application of NPs for this purpose. In a study, a colorimetric assay was designed for the detection of MERS-CoV via colorimetric changes of Au NPs [112]. The assay was based on the changes in the Au NPs LSPR shift, which was detectable in ultraviolet-visible (UV-vis) spectrophotometry. For this purpose, a thiol-modified probe was designed that matched with the complementary base pairs in the upstream of the E protein gene (upE) and open reading frames (ORF) 1a on MERS-CoV. In the presence of the virus, dsDNA was formed via hybridization of probe and target, which turned into a disulfide

induced self-assembly that could prevent Au NPs aggregation after salt addition. The color and UV-Vis spectral changes after LSPR shift due to the NPs aggregation were then interpreted. Another NP-based platform for the detection of MERS-CoV along with two other viruses was designed [113]. In this sensor, pyrrolidinyl peptide nucleic acid (acpcPNA) probes with the complementary sequence with that of the MERS-CoV genome were used. These probes also had a positive charge to be able to aggregate the citrate-stabilized Ag NPs. In the presence of the complementary nucleic acid, hybridization of acpcPNA and target prevented Ag NPs aggregation. Yet in the absence of the target or the mismatching nucleotides, the acpcPNA-induced Ag NPs aggregation led to a detectable color change. The proposed sensor enabled the rapid, sensitive, and specific detection of the viral genome. Apart from colorimetric assays, Au NPs were used in an electrochemical immunosensor for the detection of HCoV and MERS-CoV [114]. In this biosensor, carbon electrodes were modified with Au NPs, which were immobilized with HCoV and MERS-CoV S protein antigens. Followed by the addition of varying concentrations of viral antigens with a constant concentration of specific Ab, the voltammetric response was detected. The peak current was associated with free antigen concentration since, in the absence of free antigen, binding of Ab to immobilized antigen could lower the electron transfer efficiency leading to a decrease in the current. Recently, a dual functionalized biosensor was designed based on the LSPR and plasmonic photothermal effect (PPT) of Au nanoislands (NIs) chips [115]. The AuNIs were functionalized with complementary DNA receptors of specific regions in the SARS-CoV-2 genome including RdRp-COVID, ORF1ab-COVID, and E genes. In the presence of the virus, the hybridization of target and receptors were detectable via LSPR response. The important feature of this biosensor was the thermoplasmonic enhancement, which took advantage of the PPT effect of AuNIs to increase the precision of the biosensor. The plasmonic heat generated by excitation of

AuNIs at the specific wavelength, increased the kinetics of hybridization of fully-matched sequences, while disabled the hybridization of closely related sequences with some mismatch points. This PPT effect enables the discrimination of similar genes from the specific target with a limit of detection (LOD) concentration of 0.22 pM. Another recent study developed a rapid selective naked-eye method that enabled the detection of the SARS-CoV-2 with LOD of 0.18 ng/μL of RNA [116]. In this method, Au NPs were capped with thiol-modified antisense oligonucleotides (ASOs) specific for the N gene of SARS-CoV-2. Agglomeration of Au NPs in the presence of the target RNA led to their LSPR change. At this stage, the addition of RNaseH by detaching the RNA strand from the hybrid facilitated further agglomeration of Au NPs enabling the visual detection of the precipitation (Figure 5). The mentioned NP-based detection tools with their promising results accentuate the great potential of NPs to be deployed in diagnostic approaches.

4.2 Serologic detection

Serologic detection methods for identification of immune response to SARS-CoV-2 are not the gold standard in disease detection because antibody responses vary based on the patient's condition, age, and immune system. Besides, immunoglobulin M (IgM) and immunoglobulin (IgG) antibodies reach the detectable threshold over days to weeks after the onset of the disease, so early tests might lead to false-negative results [117]. Moreover, these tests also might yield false-positive results in analogous infections [117]. Yet the research in this field is dynamic for exploring innovative diagnostic tools that can easily and rapidly detect antibodies. There have been recent advances in NP-based lateral-flow assays for the detection of IgG and IgM antibodies against SARS-CoV-2. Chen et al., 2020, developed a lateral flow immunoassay (LFIA) that used lanthanide-doped polystyrene nanoparticles (LaNPs) to detect IgG against SARV-CoV-2 in human

serum [118]. This method can be used as an alternative to the chest computed tomography (CT) method for confirmatory diagnosis of suspicious samples whose RT-PCR was reported negative. In this assay LaNPs that served as a fluorescent reporter were functionalized with mouse anti-human IgG. Also, recombinant nucleocapsid phosphoprotein (rNCp) was dispensed onto the test line to capture IgG. As the diluted sample migrated through the LFIA strip, in the presence of anti-SARS-CoV-2 IgG, the complex of mouse-anti human IgG and sample IgG formed in the test line (with rNCP), which could be detected after 10 min via the fluorescence reader. This assay could successfully detect a suspicious negative RT-PCR sample as IgG positive. In another recent study, an LFIA based on Au NPs was designed for rapid and on-site detection of anti-SARS-CoV-2 IgM antibodies [12]. For this purpose, antihuman IgG-conjugated Au NPs were used as detecting probe and SARS-CoV-2 N protein was coated in the test line (Figure 6). When the sample was added on the strip, the antihuman IgM-conjugated Au NPs could capture the antibody and the complex would flow to the test line, where the N protein could catch the IgM captured by Au NPs. Finally, the formation of (AuNP-antihuman IgM)- (SARS-CoV-2 IgM)- (SARS-CoV-2 N protein) complex in the test line could be detected. This rapid, on-site method had 100% sensitivity and 93.3% specificity. These studies show the potential application of NPs as point of care diagnostic tools based on detection of anti- SARS-CoV-2 IgG and IgM antibodies.

5. Novel applications of NPs as antiviral agent screening tools

Different role of NPs as viral antigens or antibody detecting tools was discussed so far. However, NPs can also be used in other applications such as drug discovery platforms. In an interesting study, NPs were used innovatively as a high-throughput screening platform for SARS-CoV N protein inhibitor [119]. N protein plays a pivotal role in SARS-CoV replication and is a potential target for anti-SARS therapeutics. In this study, the anti-SARS-CoV N protein activity of

polyphenolic compounds was analyzed via the optical NP-based RNA oligonucleotide(RO) biochip system. For this purpose, SARS-CoV N protein was immobilized on a glass chip and then was treated with quantum dots (QDs), which were conjugated with RO sensitive to SARS-CoV N protein to enable the binding of RO and N protein. Then the inhibitor was spotted on the chip and followed by washing and unspecific binding removal the detection was performed via image analysis (Figure 7). By this low-cost method, two potent inhibitors of SARS-CoV N protein were detected. This study highlights the fact that NPs can be innovatively exploited in novel applications, which can expedite the process of drug discovery for diseases including COVID-19.

6- Conclusion and prospects

In this review, the potential ways that NPs can be deployed to provide a solution for current shortcomings in the therapeutic and diagnostic approaches in COVID-19 were discussed. Besides, very recent advances in the field of NP-based vaccines and diagnostic tools were covered.

NPMDD can be a powerful tool to address the existing challenges in treatment of COVID-19. NPs can enhance the in vivo stability of protein/nucleic acid –based therapeutics in COVID-19. Also, NPs conjugation with anti-S nAbs, can enhance viral entry inhibition both by protecting nABs from degradation and enabling the multivalent binding of the virus to NPs. The therapeutic benefits of NPMDD also include enhancing the efficacy and safety of host-directed therapeutics to stop CRS. The passive targeting of NPs to the inflammatory tissue due to the EPR effect is one promising strategy to enable effective delivery of drugs to the infected lung. The other important feature of NPs is the ability of concomitant delivery of therapeutics, which seems to add a significant benefit to COVID-19 combinational regimens. The pulmonary delivery of therapeutics by nanomaterials is another solution to decrease the safety issues caused by high concentration and frequency intake of therapeutics.

It is noteworthy that all of the mentioned applications of NPMDD need prior meticulous investigations to find the optimal characteristics and best material needed for each purpose and also by considering the potential adverse effects associated with NPs administration. For instance, the stability and bioavailability of NPs, their interactions with biologic tissue, and also SARS-CoV-2 should be thoroughly investigated, for some NPs might lack the required stability and bioavailability, particularly following oral administration, some have unintended interactions with biological tissues and environment, some have intrinsic toxicities and also off-target effects [120]. Besides, the mentioned scavenger effect of NPs also should be evaluated regarding the biological fate of the virus-NP complex.

The essential role of NPs in designing vaccines against SARS-CoV-2 is conspicuously reflected by the recent achievements in vaccine discovery of COVID-19 (table 1). In this application LNP-based platforms for delivery of mRNAs are the most effective strategy as two of the most efficacious vaccines worldwide belong to this category.

NPs can also offer great advantages in diagnostic approaches. In the condition when rapid and precise detection of disease is of great importance, NPs can be used as tools to enable the detection of the virus in simple yet accurate ways. In particular, Au NPs can be great candidates due to their LSPR and also PPT effect, where the former can enable naked-eye detection of viruses and the latter can enhance the selectivity of diagnostic platforms. NPs can also be used in serologic detection of Abs in LFIA platforms, which can be used as a point of care diagnostic materials. In addition to the mentioned applications of NPs in fighting against COVID-19, their novel applications are yet to be discovered. For instance, NPs have been used as antiviral agents screening biochip to find the inhibitor of SARS-CoV N protein [119].

To sum up, NPs can play a pivotal role in addressing the current gaps in the therapeutic and diagnostic approaches in COVI-19, yet their efficacy and safety should be meticulously evaluated in clinical studies.

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Figure captions:

Fig. 1. Advantages of NP-mediated drug delivery in COVID-19.

Fig. 2. Multivalent inactivation of SARS-CoV-2 S protein by nAb-conjugated NPs to prevent viral entry to host cells.

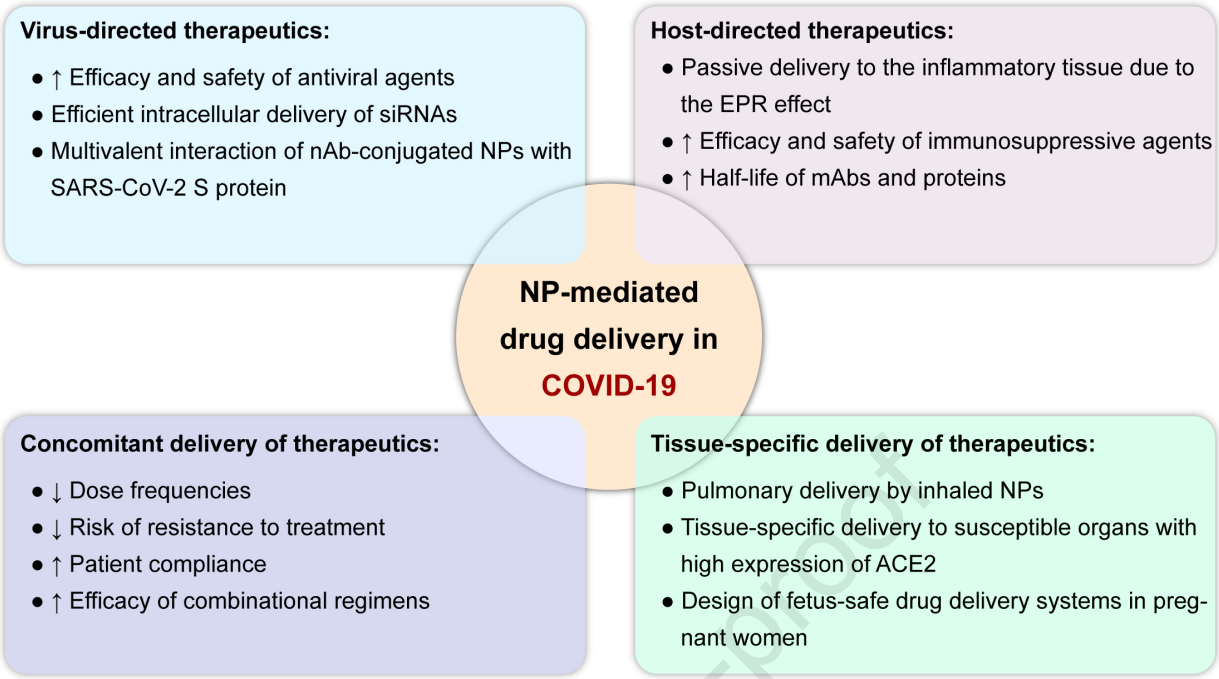
Fig. 3. Design of NP-based sVLPs with SARS-CoV-2 spike protein corona along with concomitant delivery of adjuvants as candidate vaccine platform in COVID-19.

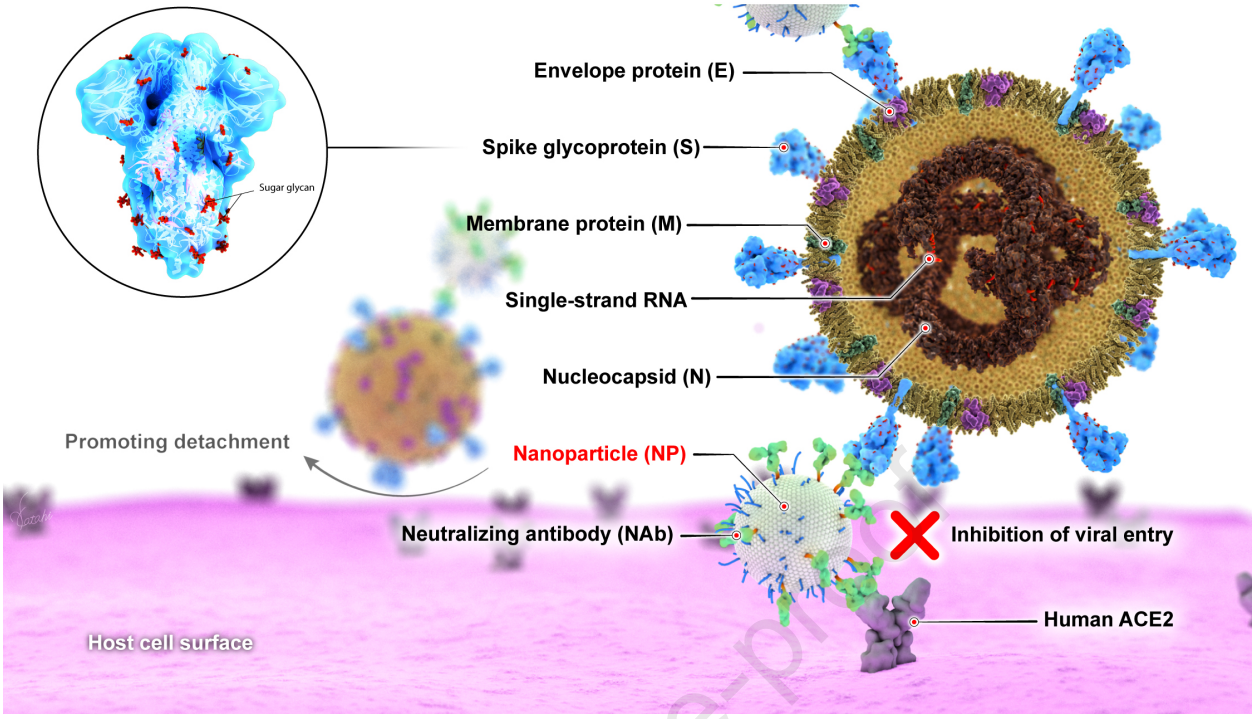
Fig. 4. Successful immunization by the administration of ARCoV against the challenge of SARS-CoV-2 in a mouse model; stimulation of T helper 1-biased cellular response and production of nAbs against SARS-CoV-2 followed by the intramuscular administration of ARCoV in mice and non-human primates. Reprinted with permission from ref [109]. Copyright 2020 Elsevier.

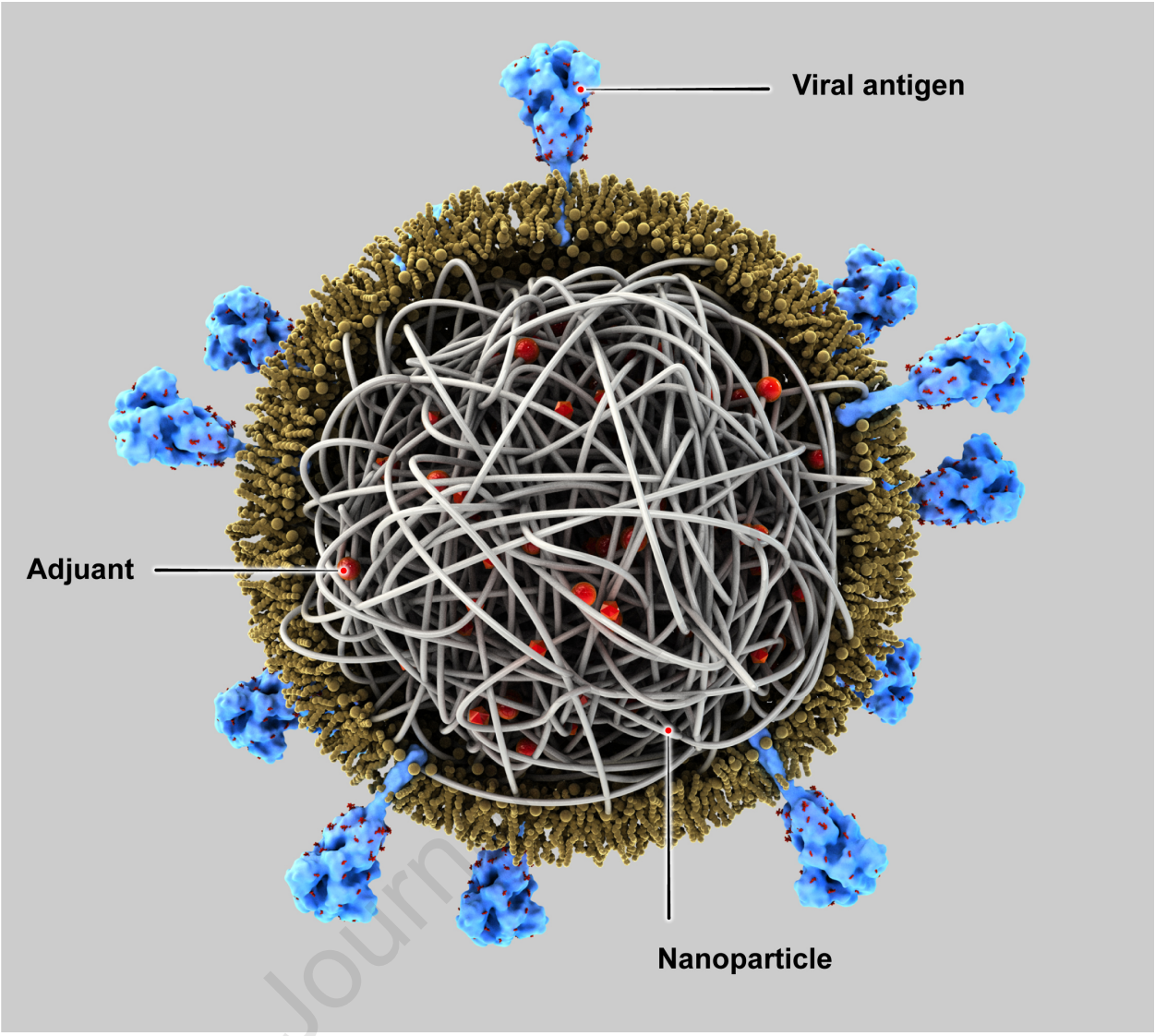
Fig. 5. Schematic Representation for the Selective Naked-Eye Detection of SARS-CoV-2 RNA Mediated by the Suitably Designed ASO-Capped AuNP. Reprinted with permission from ref [116]. Copyright 2020 ACS.

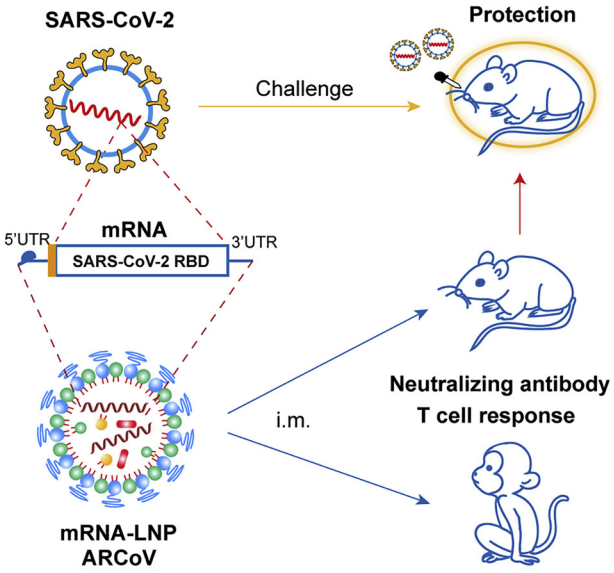
Fig. 6. Description of Operation Principle of the AuNP-LF Strip. Reprinted with permission from ref [12]. Copyright 2020 ACS.

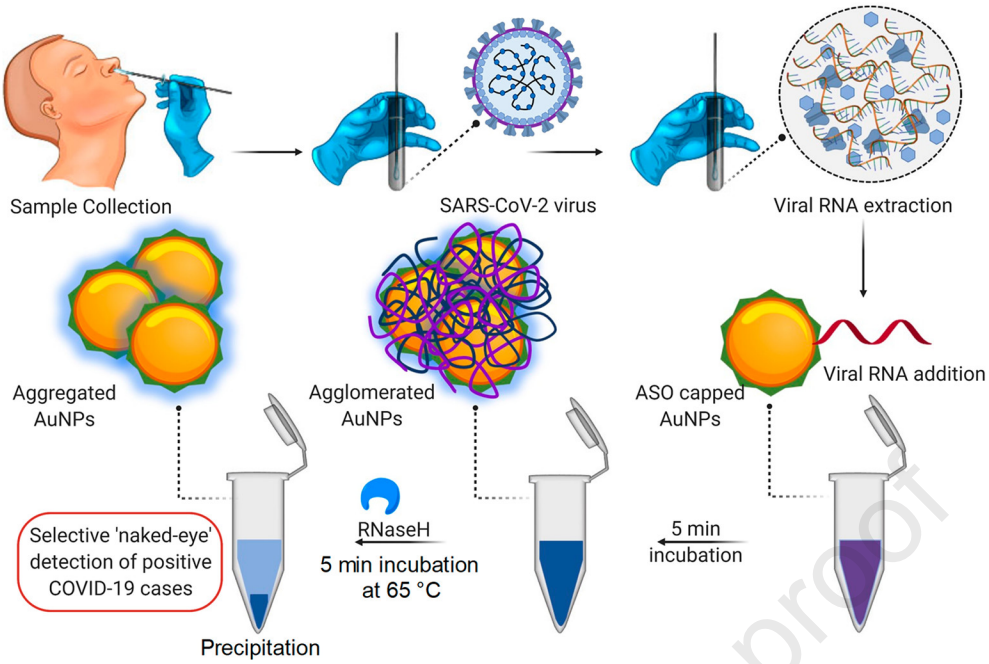
Fig. 7. A representative scheme for the inhibitor screening of SARS-CoV N protein using QDs-conjugated RNA oligonucleotide on the biochip. Abbreviations: N, nucleocapsid; SARS-CoV, severe acute respiratory syndrome-associated coronavirus; QDs, quantum dots. Reprinted with permission from ref [119].



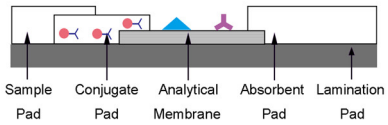



Viral antigen
Adjuant
Nanoparticle

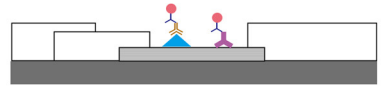




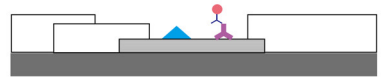
a. Structure of the AuNPs-LF strip



b. Positive detection of the AuNPs-LF strip

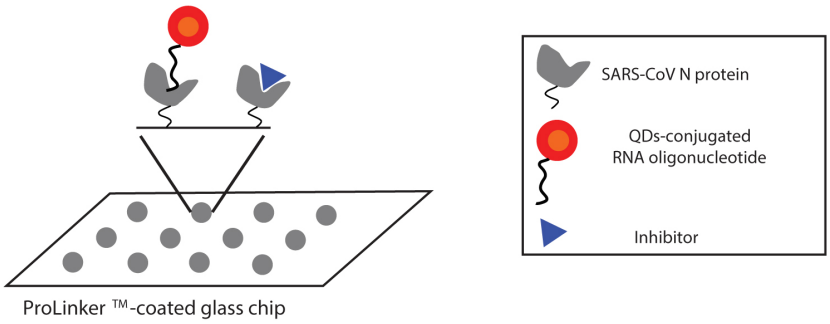


c. Negative detection of the AuNPs-LF strip



AuNPs-(anti-human IgM)
SARS-CoV-2 NP
Goat-anti-mouse IgG
AuNPs-(anti-human IgM) - (SARS-CoV-2 IgM) - (SARS-CoV-2 NP) compound
AuNPs-(anti-human IgM) - (goat-anti mouse IgG) compound

A



B